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AN ANALYSIS OF THE MOLTING PROCESS IN THE FIDDLER CRAB, *UCA PUGILATOR*¹

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The spectacular manner by which decapod crustaceans accomplish growth through molting has fascinated biologists for a long time. Considerable information has been accumulated during the past twenty years with respect to physiological changes which accompany molt. One of the results of these investigations has been the establishment of an hypothesis that hormones act as regulatory factors of certain processes associated with growth and molt.

Koller (1930) was the first to demonstrate a difference between normal and eyestalkless crustaceans in the inorganic content of exuviae; Koller used the shrimp, *Crangon (Crago) vulgaris*. Plankemann (1935) made similar observations for some other crustaceans. The first experimental demonstration that the sinus gland was involved in the regulation of molt was made for the crayfish, *Cambarus*, by Brown and Cunningham (1939); these investigators showed that acceleration of molt resulted from eyestalk removal, and that following eyestalk removal, the accelerated molting was retarded by sinus gland implants. Abramowitz and Abramowitz (1940) and Kleinholz and Bourquin (1941a) noted that eyestalk ablation in *Uca pugilator* shortened the intermolt period, but these investigators did not interpret their results as a demonstration of hormonal control of molt. A thorough discussion of crustacean hormones and their known actions is contained in a review by Brown (1952).

Alterations in contents of certain inorganic substances during the premolt and postmolt periods have been studied by Kleinholz and Bourquin (1941b) and by Guyseلمان (1950). Kleinholz and Bourquin determined the calcium content of exuviae from destalked *Uca* but presented no similar data for the exuviae from normal animals; however, the same investigators determined calcium values for the exuviae from destalked and normal *Palaemonetes* and found no appreciable difference between them. Guyseلمان found no difference between the calcium contents of exuviae of normal and destalked *Uca pugilator*, although a difference in the total

¹ This investigation is a portion of a dissertation submitted in partial fulfilment of the requirements for the Ph.D. degree in biology at Northwestern University in June, 1952. The author wishes to express his sincere appreciation to Dr. Frank A. Brown, Jr. for his advice and helpful criticism during the course of this investigation.

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ash was observed. The only other investigation pertaining to the metabolism of inorganic substances of *Uca* was that of Edwards (1950) who found that eyestalk removal effected an increase in oxygen consumption. He suggests, without adequate evidence, that the calcium content of the blood is regulated by the sinus gland and that through effecting calcium changes, the sinus gland controls oxygen consumption and other processes known to be regulated by this gland.

The study of growth and molt in *Uca pugilator* has been approached, largely, in a qualitative manner. Abramowitz and Abramowitz (1940) demonstrated that eyestalk ablation leads to substantially increased sizes of the postmolt animals. Kleinholz and Bourquin (1941a) presented data describing weight increases associated with molting in destalked animals, but no dimensional measurements were given; the destalked animals showed a weight increase of about 30%. None of their control animals molted during the experimental period; therefore, there was provided no basis for a comparison of the normal animal with the destalked.

A number of other crustaceans have been investigated with respect to growth, molt, and mineral metabolism, and the mechanisms responsible for their regulation. Baumberger and Olmsted (1928) have discussed osmotic pressure and water changes which are associated with molt in *Pachygrapsus*. Robertson (1937, 1941) has undertaken an analysis of the inorganic composition of British shore crabs; he has studied changes associated with molting, including the absorption of water at ecdysis. Drach (1939) has made excellent studies on several European forms, dealing with the molting cycle in normal animals. Numanoi (1939), Mahf (1940), Smith (1940) and Scudamore (1947) have contributed information on various aspects of the calcium metabolism in crustaceans. Kincaid and Scheer (1952) have discussed the influence of the sinus gland on the tissue composition during the intermolt cycle of *Hemigrapsus nudus*.

MATERIALS AND METHODS

Animals

The fiddler crab, *Uca pugilator*, was used for these investigations. The animals were collected during low tide in salt marshes at Chappaquoit, Massachusetts, where they occurred in great abundance. Both males and females were utilized, but the great variation in size of the large male chela rendered the males less desirable for this study. All of the animals used were collected during July and August of 1950 and 1951; wet weights and dimensions were taken only on those collected in 1951. Of 300 animals brought into the laboratory, the carapace width ranged from 1.0 to 1.9 cm., with a mean of 1.55 cm. Figure 1 shows the relationship between the cube of the carapace width and the wet weight for female animals. Conspicuous differences in coloration were noted among the animals at the time of collection and in the course of laboratory study; these differences will be discussed later.

The stock animals were kept on sea-water tables in running sea water; the floor of the table was of such a character that the water level varied over its surface from a quarter of an inch to about an inch (sufficient to cover completely an animal). For the purpose of isolating animals in sea water, No. 5 wax-coated vendor cups were punctured on two sides near their bases and arranged on the sea-water table to insure complete water coverage of a contained animal. During other experiments,

including those conducted at Evanston, Illinois, animals were isolated in 4-inch finger bowls containing sea water. The only food available to the animals during the investigations was the planktonic material which was carried in the sea water. No special attempt was made to feed the animals during the course of the work. The temperature of the sea water varied between 21° and 24° C. during the investi-

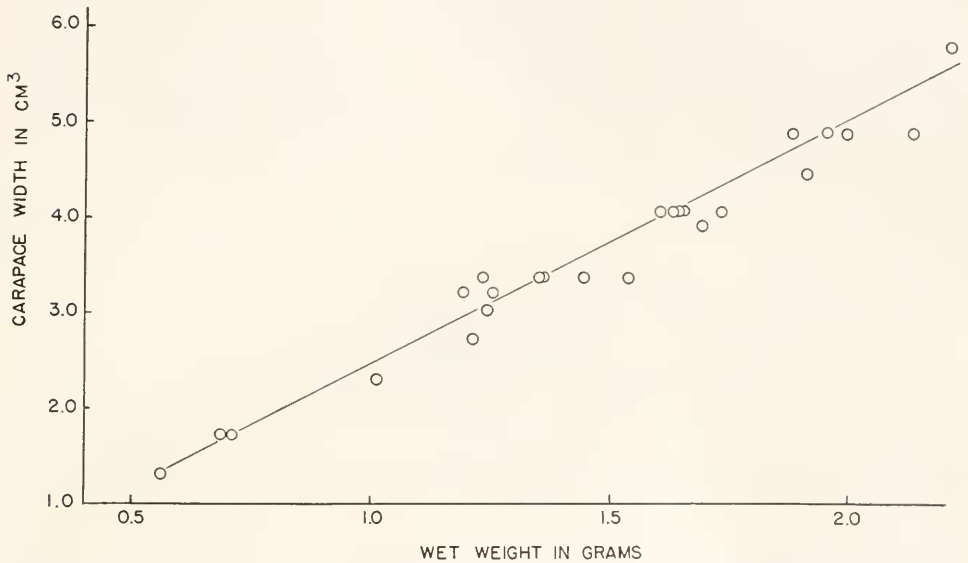


FIGURE 1. The relationship of wet weight to the cube of the carapace width in female *Uca pugilator*.

gations. Records of intensities and durations of illumination were not made; it can be stated, however, that all of the animals in the laboratory were exposed to natural and artificial light for a total period of about 20 hours daily.

Methods

1. Operations

Bilateral eyestalk removal was effected by cutting off both eyestalks at their proximal ends, insuring complete removal of the sinus gland-X organ complexes contained within the stalks.

2. Weights

All weights were determined on an analytical balance.

Wet weights were obtained by leaving an animal for three minutes on a 4-inch filter paper in a dry finger bowl; thereafter, the animal was rolled briefly in a second piece of filter paper, placed in a porcelain crucible (ca. 25 grams) and weighed. To determine whether or not this procedure gave results which were reproducible, a single animal was weighed ten times throughout the course of two hours; the mean weight was found to be 0.9541 grams, with a standard deviation of ± 0.0045 grams.

Dry weights were obtained after an animal had been subjected to one or more dorsal incisions, left in a drying oven at 90° C. for 24 hours, and then left in a desiccator for at least one hour prior to weighing.

Ash weights were ascertained after the crucible containing a dried animal had been left in a Hoskins electric furnace at 1100–1150° C. for ten minutes and then in a desiccator for two hours. All of the desiccators contained colloidal silica gel, potassium hydroxide, and a container of concentrated sulfuric acid. This prevented any hydration of the dried or ashed animal, and reduced the possibility of recombination of the ash with atmospheric carbon dioxide. The ashing temperature was sufficient to convert, quantitatively, pure calcium carbonate to calcium oxide and carbon dioxide, and to decompose pure tricalcium-tertiary-phosphate to a complex containing 0.79 grams of ash per 1.00 gram of reagent phosphate.

3. Chemical analyses

Ionic calcium content was determined by a modification of the "Versenate" titrational method (Guyseelman, 1951).

Phosphorus content was determined by the Fisk-Subbarow method; the measurement was made on a Coleman Junior Spectrophotometer.

Carbon dioxide was measured by treating a dried animal with 4 N hydrochloric acid in such a manner that the resulting gaseous expansion occurred in a closed system of adjustable volume. With this apparatus, the volume of gas generated was readily measured; the error was found to be less than 2% when 0.090 grams of pure calcium carbonate were used.

Terminology applied to the molting process

Other investigators have employed various terminologies to describe the various phases and events associated with the molting cycle. The following classification of phases will be used for this work:

1. Intermolt—that period, if any, during which there is a maintenance of a steady-state of the hardened cuticle. It is extremely variable in duration under certain conditions, and may even be lacking in zooecial stages.

2. Premolt—that period during which certain processes lead to epidermal separation.

3. Molt—a time of epidermal separation from the old cuticle, of the formation of a new cuticle, and of the subsequent shedding (ecdysis) of the old cuticle (exuvia).

4. Postmolt—a period of cuticular expansion and hardening, gradually leading either to the intermolt or premolt condition.

EXPERIMENTS AND RESULTS

A. Premolt

1. Color changes

It was stated earlier that differences in coloration were observed in freshly collected animals. Since it had been reported that certain changes in pigmentation were a visible indication of active processes leading to ecdysis in some of the

Brachyura (Drach, 1939), the differences in coloration in *U. pugilator* were studied with reference to such a possible relationship.

Cursory observations made during the summer of 1950 on animals retained for some time in the laboratory indicated that the gross pigmentary pattern of an animal in which ecdysis was to occur in two or three days differed from that of individuals ordinarily collected in the field. The former animals were observed to be blue-gray in color, in contrast to the specific patterns of brown, light brown, and purple possessed by the latter and giving the species the colloquial name, "calico backs." However, there seemed to be no indication of any progressive gross color changes preceding the adoption of the blue-gray color which could be regarded as an indication of approaching ecdysis.

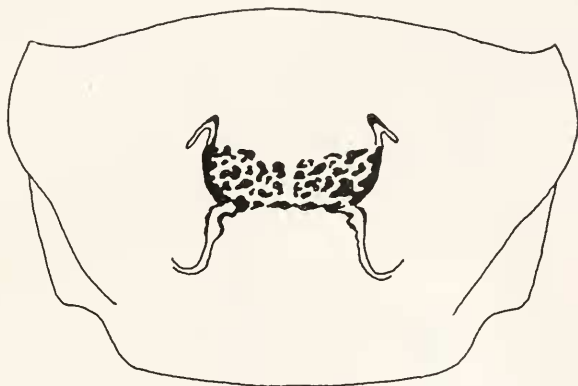


FIGURE 2. Dorsal aspect of the carapace of *Uca pugilator* showing the dorsal crest. The appendages have been removed.

In a group of animals collected on July 29, 1951, the type of coloration in a small region of the carapace served as a means of segregating the animals into three separate groups. Figure 2 is a line drawing of the carapace, showing this distinctive region which will be referred to subsequently as the "dorsal crest." The three groups showed the following differences in the dorsal crest:

- Group 1: white patterns on a brown field (Fig. 3a)
- Group 2: pale orange and white patterns on a brown field (Fig. 3b)
- Group 3: indistinct gray and pale orange patterns on a gray-brown field (Fig. 3c)

During the course of daily observations, a fourth pattern of coloration appeared within animals of group 3. This fourth group had the following characteristics:

- Group 4: yellow-orange patterns on a light gray-brown field (Fig. 3d)

Similarly, animals now in group 4 were the source of a fifth pattern which was observed:

- Group 5: light yellow patterns on a light blue-gray field (Fig. 3e). Animals in this condition had a gross blue-gray color and were almost identical with those observed in 1950 which were within a few days of ecdysis.

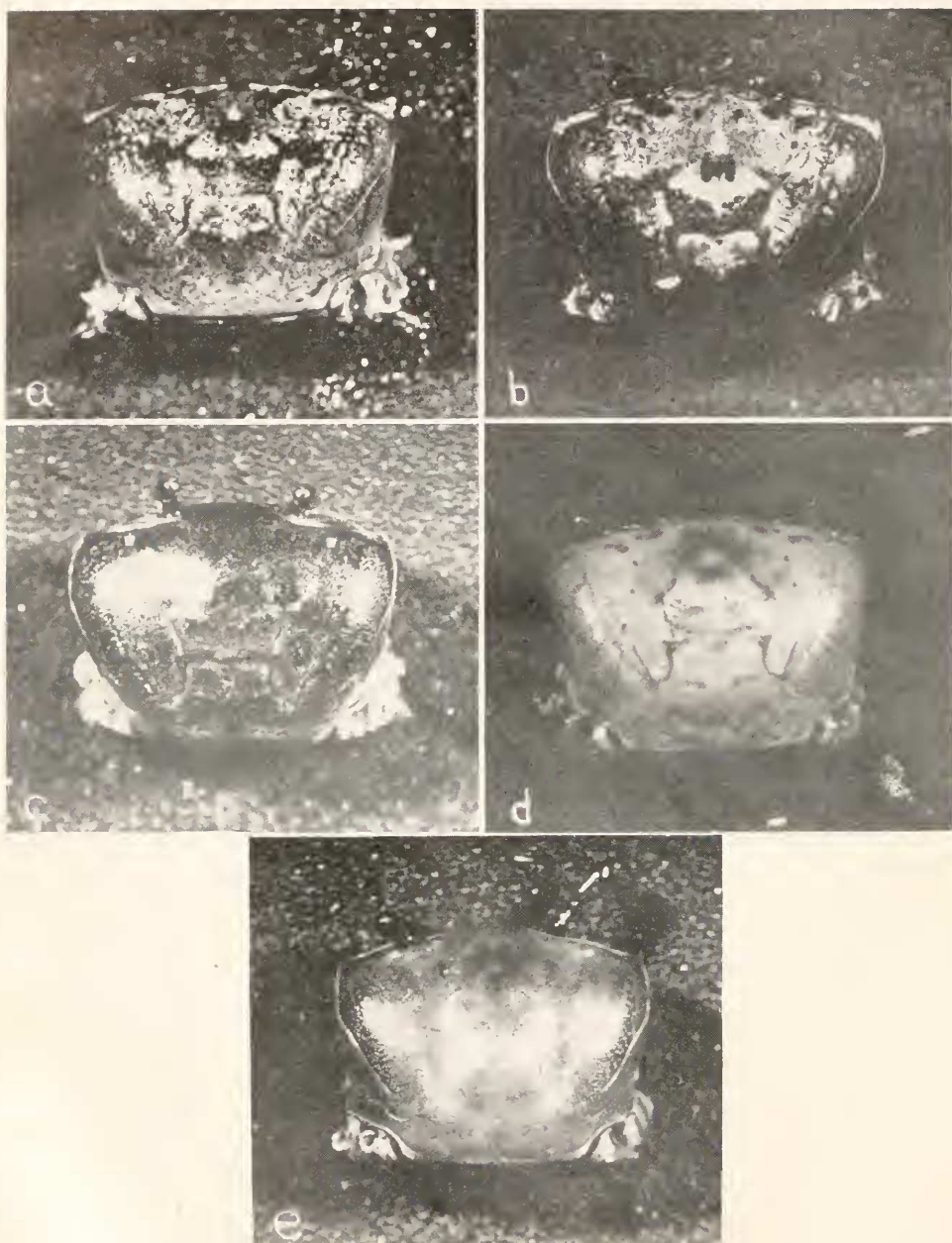


FIGURE 3. Photographs of normal *Uca pugilator* in each of the five stages: (a), stage one; (b), stage two; (c), stage three; (d), stage four; (e), stage five.

Table I shows changes in the number and percentages of animals contained within each of the five groups over a six-day period. It appeared obvious from these data that a progressive change in coloration was occurring in a direction from group 1 to group 5, but evidence was incomplete in that no single animal was seen to make a complete transition from 1 to 5.

Figure 4 shows the changes in group status of a single animal initially in group 1 over the course of 23 days. That this transition occurred in the direction from 1 to 5 was now established beyond doubt. Consequently, the term "stage" was now adopted in place of "group." Furthermore, the length of time that an animal remained in each of stages 2 through 5, under these laboratory conditions, was established. The duration of stage 1 was not determined but the coloration characteristics of an animal immediately following ecdysis are those of a stage 1 animal, as is shown by Figure 5. During a period of a week or two after ecdysis, the maximum time that such animals were observed, no visible changes in coloration occurred. There

TABLE I
Inter-group transition in normal U. pugilator

Date	Percentage of animals in each group					Number of animals
	Group:					
	1	2	3	4	5	
7-29-51	24 ^c _c	27 ^c _c	49 ^c _c	0 ^c _c	0 ^c _c	56
7-31-51	24	26	50	0	0	56
8- 1-51	24	25	46	5	0	56
8- 2-51	23	24	24	23	6	52
8- 3-51	15	16	27	26	16	52
8- 4-51	5	18	28	36	13*	50

* Of the seven animals in group five, two underwent ecdysis, three died, and two were sacrificed for inorganic analyses.

is nothing to suggest that any coloration change occurs between ecdysis and the premolt period. Therefore, stage 1 is presumed to extend throughout the inter-molt period.

No attempt was made to interpret these changes in coloration at the histological level; however, it can be shown that this transition is correlated with the process of formation of the new cuticle and its separation from the old exoskeleton, and probably also with the partial resorption of the old cuticle. Figure 5a shows a stage 5 animal with a portion of the carapace removed; the new cuticle (with stage 1 characteristics) has separated completely from the old cuticle. Similar examinations were made on animals in other stages; only in 3, 4 and 5 was cuticular separation clearly evident. Considerable variation, however, existed in stage 3 animals with regard to the completeness of cuticular separation; in some cases it was still attached, at least in part, to the carapace while in others it had separated.

During the remainder of the summer and fall of 1951 three additional series of animals were isolated and examined daily for progressive stage changes. The re-

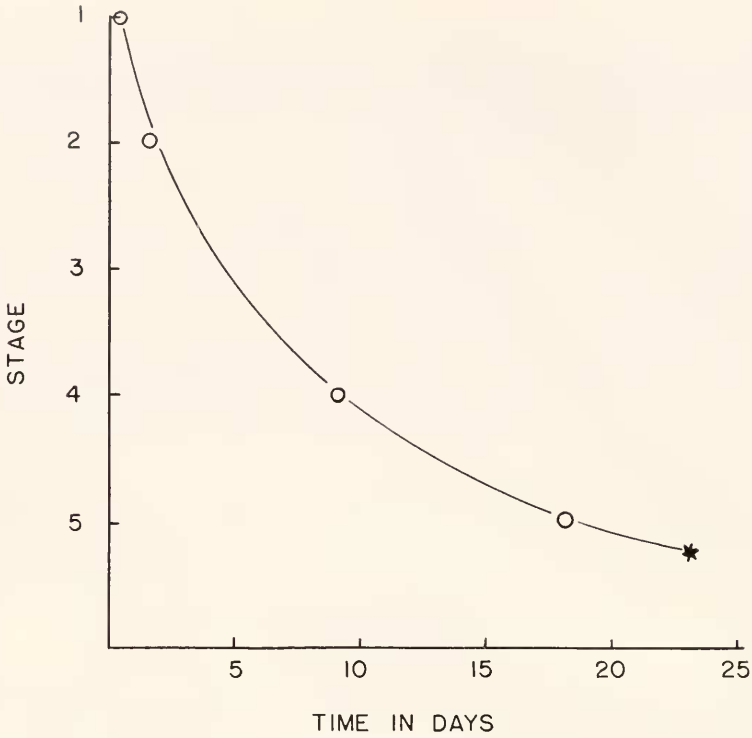


FIGURE 4. Interstage transition for a single, female *Uca pugilator* from stage one until ecdysis; *ecdysis.

sults of these investigations are given in Table II. It appears evident from these results that interstage transition does not occur at the same rate at all times of the year. This is further substantiated by the decreased incidence of ecdysis in series B and C animals.

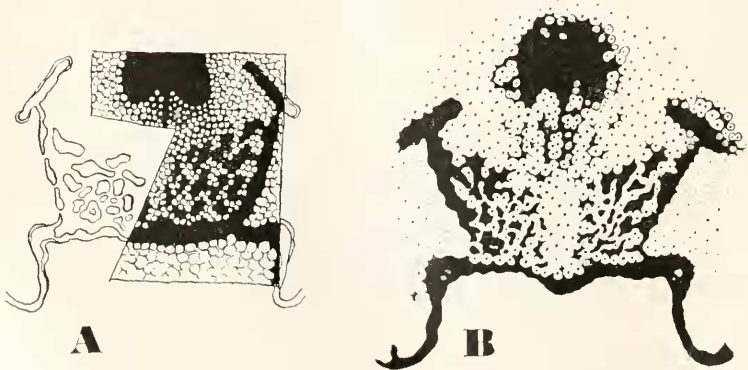


FIGURE 5. Carapace coloration of *Uca pugilator* in stage five (a) showing portion of new cuticle, and carapace coloration in the postmolt condition (b).

During these investigations of the changes in coloration of the carapace in normal animals prior to ecdysis, an attempt was made to determine whether or not a similar series of changes took place in destalked animals. Observations made during the summer of 1950 on destalked animals seemed to indicate that there was little or no difference in gross coloration between animals that had been destalked for a day or two and animals which were within a few hours of ecdysis. Furthermore, it had been shown that one of the effects of bilateral eyestalk removal on *U. pugilator* was a paling of the carapace (Abramowitz and Abramowitz, 1940). Daily observations

TABLE II

Inter-stage transitions at different times of the year during five-day periods. Percentages represent the distribution at the beginning and at the end of five-day periods

Stage	Group A 7-31-51 to 8-4-51		Group B 8-11-51 to 8-15-51		Group C 10-22-51 to 10-27-51	
1	24%	5%	35%	13%	24%	25%
2	26	18	30	27	64	66
3	50	28	27	21	8	6
4	0	36	6	3	0	0
5	0	13	2	0	0	0
Total no. of animals observed	100	64	56	50	30	30
Total no. of animals that molted		2		0		0

on a series of animals destalked July 28, 1951, showed no indication of transitional color changes; in all instances these animals assumed a light gray-tan color and retained to some extent their previous dorsal crest coloration until the time of ecdysis.

2. Inorganic constituents of normal animals

In view of the fact that visible alterations in coloration occurred in normal crabs antecedent to ecdysis in such a regular manner that the approximate time of the subsequent ecdysis could be predicted, a method was provided by which chemical analyses of animals could be made at determinable periods prior to ecdysis. It will be recalled that the staging by coloration had not been perfected in time for the studies made in 1950; therefore, the analyses made at that time could be correlated only with three phases: the first including stage 1 and 2; the second, 3; and the last, 4 and 5. In the analyses to be described in this section, stage 1 animals were restricted to those which, as far as could be determined, had completed their postmolt hardening of the exoskeleton.

Calcium

It has been reported that calcium constitutes 16.3% of the dry weight of a freshly killed *U. pugilator* (Kleinholz and Bourquin, 1941b). This value is based on the average obtained with five animals. Table III contains figures for the dry weight, ash

TABLE III
Inorganic constituents of normal U. pugilator

Animal no.	Sex	Width (cm.)	Stage	Dry weight (gms.)	Ash weight (gms.)	Calcium (gms.)	Calcium as % of dry weight
C99	F	1.6	1	0.7909	0.1627	0.072	9.1
C84	F	1.6	3	0.4903	0.1420	0.058	11.9
B16	F	1.6	3	0.5283	0.1929	0.048	9.0
C42	F	1.65	2	0.6471	0.1703	0.078	12.0
C101	M	1.5	5	0.7754	0.2600	0.084	10.8
C102	M	1.65	1	0.7628	0.3224	0.106	13.8
C103	M	1.7	3	0.8124	0.3048	0.114	14.0
C31	F	1.65	1	0.6010	0.1586	0.072	11.9
D29	M	1.8	2	0.5625	—	0.078	15.4
C111	M	1.0	2	0.1415	—	0.020	14.2
10	M	2.2	1-2	0.7394	0.2704	0.084	11.4
55	M	—	1-2	0.6737	0.2150	0.128	19.0
56	M	—	1-2	0.6483	0.2110	0.101	15.4
57	M	—	1-2	0.8493	0.2662	0.139	16.3
8	M	—	4-5	1.1359	0.4029	0.188	16.5
58	M	—	1-2	0.6518	0.2358	0.096	14.7
59	M	—	1-2	0.6975	0.2290	0.122	17.5
43a	M	—	3	0.7052	0.2382	0.140	19.8
C57	M	—	1-2	0.7569	0.2515	0.124	16.3
6	M	—	4-5	0.8175	0.3058	0.176	20.3

weight, calcium, and calcium as the percentage of dry weight for 20 animals. It will be seen that the amount of calcium ranges from 9.0% to 20.3% of the dry weight, with an average of 14.4% and a standard deviation of $\pm 3.4\%$. While 9.0% is the minimal value for an animal in stage 1, a value of 10.8% is found for an animal which appears to be within a day or two of ecdysis. From these data it would seem that there is no correlation between the stage of an animal and the amount of calcium it contains. A value of 9.0% would seem to be the minimal amount occurring in a fully hardened animal.

The most abundant forms of inorganic calcium in decapod crustaceans are carbonate and phosphate. Table IV shows the proportions in which these salts occurred in four completely hardened animals. Calcium carbonate constituted about 27% of the dry weight while calcium phosphate comprised about 9%. The ratio

TABLE IV
Carbonate and phosphate contents of normal U. pugilator

Animal	Sex	Width (cm.)	Stage	Dry weight (gms.)	Calcium carbonate		Calcium phosphate	
					grams	% of dry weight	grams	% of dry weight
C112	F	1.2	3	0.191	0.055	28.8	0.022	11.6
C111	M	1.0	2	0.141	0.033	23.5	0.010	7.2
D29	M	1.8	2	0.562	0.172	30.5	0.049	8.8
D31	F	1.65	1	0.560	0.140	25.0	—	—

of the carbonate to the phosphate in animals under these conditions appears, therefore, to be approximately 3:1. Data are not available for all of the five stages in the molting cycle with respect to carbonate and phosphate contents. However, as will be evident later, studies of ashed animals give data which permit one to determine whether any significant alteration in this 3:1 ratio has occurred. These later data were obtained from animals representing all five stages.

Ash

At the temperature employed for the ashing of animals, all organic matter was destroyed, calcium carbonate was quantitatively converted to calcium oxide, and calcium phosphate was decomposed to a mixture of its oxides. An average value of 32.7% was obtained for 17 animals in all of the stages; values ranged from 20.6% to 42.2% for animals in stage 1, with a mean of 29.7%. Animals in stages 2 through 5 gave values of from 29.0% to 37.4% with a mean of 33.9% and a standard deviation of $\pm 2.6\%$. The greatest variation is found in stage 1 animals, all of which, however, appeared to have completely hardened exoskeletons.

Water

The water content of animals in stages 1 through 5 was found to be 67.9% with a standard deviation of $\pm 4.7\%$, values ranging from 57.1% to 74.0% for 13 animals. No significant variation in water content of hard-shelled animals was observed through the five stages. The variation in water content of stage 1 animals during the period of the hardening of the exoskeleton will be treated later. It should be mentioned that the average value of 67.9%, found in these analyses, was obtained with animals that had been kept in running sea water. For a period of ten days, relatively low weights were obtained between 7 P.M. and 8 P.M., while higher weights were found during the morning hours. Figure 6 contains values for the wet weights of an animal during the course of three days prior to ecdysis. It will be seen that during the last two days prior to ecdysis the fluctuations appear arrhythmic, in contrast to a diurnally rhythmic tendency which had been noted during the first seven days of observation. Furthermore, the amplitude of the fluctuations decreases as the animal approaches ecdysis. Immediately after ecdysis the minimal daily weight was obtained at about 6 P.M., while the maximal weight was obtained at about 6 A.M. This aspect of postmolt weight changes will be discussed in a later section. Animals kept in distilled water for 24 hours showed arrhythmic fluctuations and the amplitude of the fluctuation was considerably reduced as compared to the changes in the animal shown in Figure 6. No weight fluctuations of a rhythmic character were observed in animals kept in finger bowls of sea water in the experiments performed in Evanston, Illinois in October and November. The fluctuations observed were, however, essentially of the same amplitude as those reported for animals at the Marine Biological Laboratory during the summer.

3. Inorganic constituents of destalked animals

Calcium

No appreciable difference could be detected between the calcium contents of normal and destalked animals. For a group of animals which had been destalked for

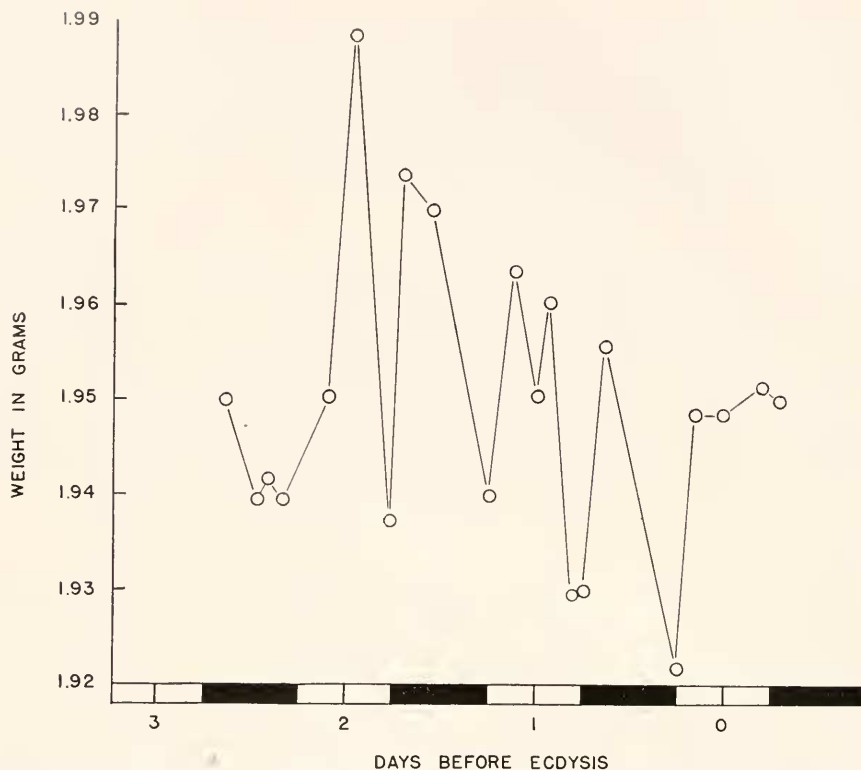


FIGURE 6. Weight changes in single, normal, female *Uca pugilator* before ecdysis. The black bar indicates a period from 6 P.M. to 6 A.M.

three, five, seven, and fourteen days, the mean percentage of calcium was found to be 16.4% with a standard deviation of $\pm 2.1\%$, values ranging from 12.9% to 18.7%. These values are well within the range found for normal animals. While calcium is not stored in the hepatopancreas of *U. pugilator* to any appreciable extent (less than 0.5% of the total dry weight in normal animals), one of the effects of bilateral eyestalk removal has been shown to be the complete depletion of calcium from the hepatopancreas within five days after the operation (Guyselman, 1950).

Ash

The ash, expressed as percentage of dry weight, was found to be 36.1% with a standard deviation of $\pm 4.7\%$, values ranging from 30.6% to 45.5%. Again, the average value is not significantly different from that found for normal animals. That certain changes in the inorganic composition do occur following destalking will be shown by a comparison of the exuviae of destalked animals, cast at various times after destalking, with those of normal animals.

Water

The water contents of destalked animals, prior to ecdysis, can be treated only in an indirect manner. Wet weights were determined for five destalked animals

during periods of six to ten days. The results are given in Table V. It does not appear likely from these data that any significant changes occurred in the water contents during this period. There may be a slight increase in weight for the first few days, but after one week the weights are essentially the same as the initial weights.

Three destalked female animals, weighed at the end of seven, eight, and twelve days after destalking, showed that there was no increase in wet weight when compared to normal animals of the same carapace width. One of these animals, 1.8 cm. in carapace width, weighed 2.06 grams on the seventh day after destalking; another, having the same carapace width, weighed 2.05 grams on the twelfth day after de-

TABLE V
Wet weights (in grams) of destalked U. pugilator

No. of days after destalking	Animal number				
	D4	D5	D6	D7	D18
1	1.682	1.588	1.238	1.848	0.704
2	1.703	1.617	1.242	1.872	0.703
3	1.739	—	—	1.904	—
4	1.726	1.636	1.260	1.921	0.708
5	1.685	—	1.244	1.890	—
6	1.694	—	—	1.878	—
7	—	1.597	1.244	1.898	0.723
8	—	—	1.252	1.924	—
9	—	—	1.242	1.892	—
10	—	—	—	1.884	—

stalking. The third animal, 1.9 cm. in width, weighed 2.64 grams eight days after destalking. It will be seen, by inspection of Figure 1, that these values are well within the range of weight variations that are found in normal animals.

B. Molt

One of the processes which characterizes the initial phase of molt is the separation of the epidermis from the cuticle. It will be remembered that this epidermal separation was first noticed in animals which were in stage 3. The period from stage 3 to the termination of molt was from 15 to 20 days in those animals which were examined during the early part of August. The duration of stage 3 appeared to be about 6 days at this time. Therefore, assuming that molting activity of the epidermis commences during the latter part of stage 3, an animal would be engaged for about 10 days in visible aspects of the molting process, a process which is terminated by the abrupt shedding of the old exoskeleton.

Bilateral eyestalk removal, thereby eliminating both sinus gland-X organ complexes, will induce molt in *U. pugilator* (Abramowitz and Abramowitz, 1940). Table VI contains data for a group of animals which were destalked on July 28, 1951; all the animals were staged before destalking. These results, despite the small number of animals used and the variability observed, nevertheless suggest that there is an

inverse correlation between the stage number of an animal at the time of destalking and the time elapsing between destalking and ecdysis. For animals in stages 1 and 2, 18 days elapsed; for stage 3, 14 days elapsed; and for stage 4, 10 days elapsed. It is especially noticeable that stage 3 animals show a dichotomy with respect to the interval between destalking and ecdysis, being of the order of either 6 to 8 days, or 15 to 25 days.

The actual process of shedding the old exoskeleton is relatively rapid; normal animals were observed to complete the casting process in about 15 minutes. Such animals showed little motor activity for several hours prior to ecdysis; occasionally these animals and ones in stage 4 were observed to exhibit non-locomotor move-

TABLE VI

Relationship of color-stage in destalked U. pugilator to interval between destalking and ecdysis

Animal no.	Sex	Carapace width (cm.)	Stage at time of destalking	Number of days after destalking that ecdysis occurred
11	F	1.5	1	15
27	F	1.6	1	18
15	F	1.7	2	19
14	F	1.7	2	21
22	F	1.6	2	17
8	F	1.5	2	14
17	M	1.9	2	14
6	M	1.6	2	27
35	M	1.5	3	18
45	M	1.6	3	14
41	M	1.7	3	27
5	M	1.7	3	15
13	F	1.3	3	8
9	F	1.5	3	6
40	F	1.5	3	12
47	F	1.6	3	14
10	F	1.6	3	18
37	F	1.6	3	18
30	F	1.7	3	7
46	F	1.7	3	13
25	F	1.7	4	15
44	F	1.4	4	5

ments of the walking appendages. This activity was never observed in animals in stages 1, 2, and 3.

The first indication of ecdysis was a separation at the cephalothoracico-abdominal junction. This was followed by a splitting along the lateral margins of the exoskeleton; as the posterior and lateral margins of the carapace split, the animal underwent active muscular movements, gradually withdrawing itself from the exuvia.

Many of the destalked animals did not free themselves completely from the exuviae, certain anterior portions of the alimentary tract remaining attached. This apparent failure of destalked animals to emerge successfully was also observed by Abramowitz and Abramowitz (1940).

1. Uptake of water

Table VII gives the weights of three animals prior to ecdysis, the weights within 15 minutes after emergence, the weights of the cast exuviae, and the increase in weight due to water absorption. In addition, the table includes the weights of the freshly molted animals and the water absorbed, expressed as percentages of the weights immediately prior to ecdysis. It will be seen that the amount of water absorbed at ecdysis is inversely proportional to both weights and carapace widths of the animals before ecdysis. It was found that the product of the cube of the carapace width and the weight of the water absorbed (expressed as the percentage of the weight prior to ecdysis) was relatively constant; for the animals indicated in Table VII, a mean product of 170.0 with a standard deviation of ± 4.7 was obtained. This indicates that the increase in water content at ecdysis appears to be a function of the volume of an animal, with the smaller animals absorbing a higher percentage than the larger ones.

TABLE VII

Changes in wet weights associated with ecdysis in normal, female U. pugilator

Animal no.	Width (cm.)	W1	E1	W2	W3	E1 as % of W1	W2 as % of W1	W3 as % of W1
		(grams)						
C11	1.7	1.960	0.899	1.740	0.679	45.8	89.0	34.6
51F	1.6	1.619	0.819	1.458	0.658	50.6	91.8	40.6
5F	1.5	1.540	0.868	1.494	0.822	56.4	96.8	53.4

W1 = wet weight before ecdysis

E1 = wet weight of exuvia

W2 = wet weight after ecdysis

W3 = W2 - (W1 - E1) = weight of water absorbed at ecdysis

It was demonstrated earlier that the water content of animals before ecdysis is about 68%. The water content of an animal 15 minutes after ecdysis is approximately 84%, values of 84.5% and 84.1% having been obtained with two animals. Although this value of 84% has been derived from direct determinations using only two animals, it is readily possible to calculate the water contents of the three animals described in Table VII immediately following ecdysis. When this is done, the three values are 82.3%, 89.6%, and 83.1%. The average for these values is 85.0%, which is in close agreement with those obtained by direct determinations.

Sufficient data are lacking for destalked animals with respect to water uptake at ecdysis. However, exploratory research seems to indicate that the increase in weight accompanying ecdysis is approximately the same as that found for normal animals. Kleinholz and Bourquin (1941a) determined the percentage weight increases in destalked *U. pugilator* following ecdysis; however, initial and final weights were not given. They state that of six animals in the 1.6 to 2.0 gram size-class, the average percentage gain in weight after molting is $29.83 \pm 7.20\%$. This value would indicate that, under certain conditions, destalked animals may absorb even less water than normal animals at ecdysis.

2. Weight changes

Figures 7a and 7b show the weight changes in two postmolt animals during a period of five days. It will be seen that at the end of this period the animals have not regained the weight they had prior to ecdysis. None of the animals was weighed at a later time than ten days after ecdysis.

An inspection of the plotted weights in Figures 7a and 7b suggests, again, a rhythmical variation, with the greatest weights occurring at about 6 A.M., and the

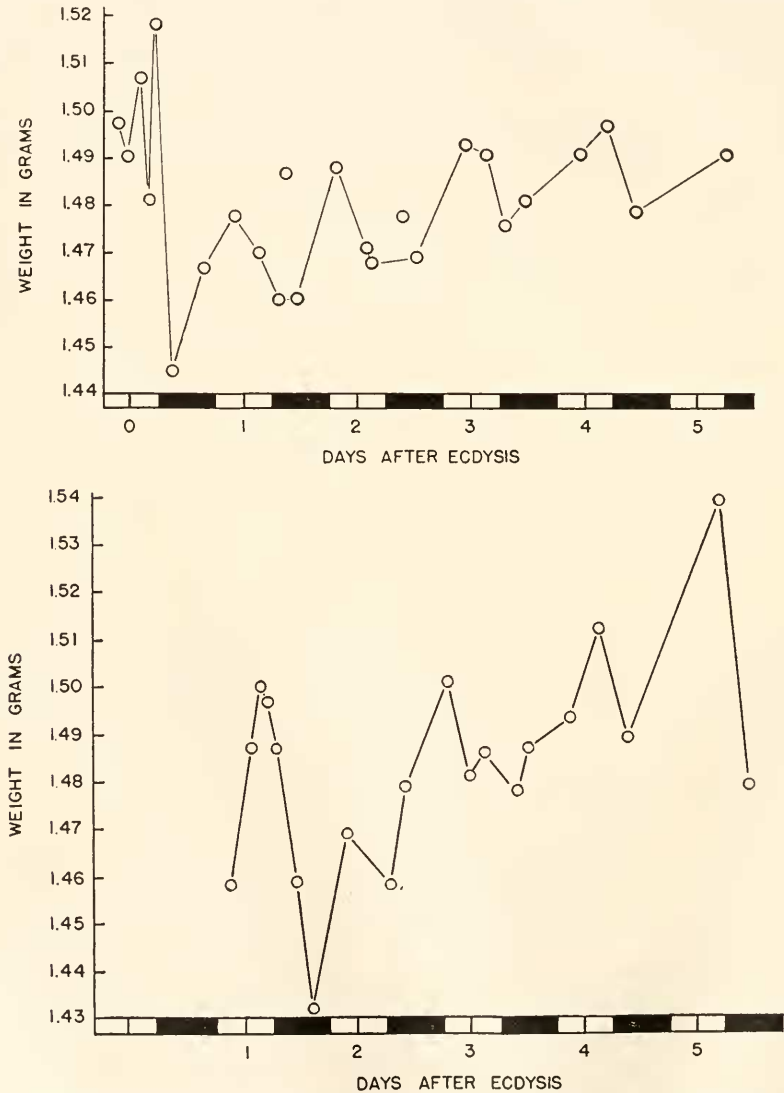


FIGURE 7. Weight changes of two, normal, female *Uca pugilator* (a and b) following ecdysis. The black bar indicates a period from 6 P.M. to 6 A.M.

TABLE VIII

Changes in carapace width in normal and destalked U. pugilator following ecdysis

Animal no.	Condition	Width before ecdysis (cm.)	Days after ecdysis that new dimensions were taken	Width after ecdysis (cm.)	Percentage increase in width
40	Destalked	1.5	0	1.6	6.7
14	Destalked	1.7	2	1.8	5.9
41	Destalked	1.7	3	1.8	5.9
35	Destalked	1.5	5	1.6	6.7
13	Destalked	1.3	7	1.35	3.8
49	Destalked	1.6	8	1.8	12.5
25	Destalked	1.7	8	1.9	11.8
15	Destalked	1.5	18	1.8	20.0
A26	Normal	1.6	1	1.6	0
C105	Normal	1.3	2	1.3	0
51F	Normal	1.6	5	1.6	0
5F	Normal	1.5	5	1.5	0
124	Normal	1.3	7	1.3	0
C11	Normal	1.7	7	1.7	0

smallest at about 6 P.M. The rhythmical consistency of the fluctuations in weights which was obtained for these animals suggests strongly that some factor other than error inherent in the technique in weighing is responsible for this orderly fluctuation.

3. Dimensional changes

Of primary interest is the fact that none of the three normal animals, discussed above in Table VII, showed any change in carapace width during the ten-day period following ecdysis; all three retained their pre-ecdysis dimensions. Three additional animals, the weights of which were not obtained (hence they received relatively little handling), similarly failed to show an increase in width.

TABLE IX

Inorganic constituents of normal and destalked, postmolt U. pugilator

Animal no.	Condition and days postmolt	Dry weight (gms.)	Ash		Calcium	
			(gms.)	% of dry weight	(gms.)	% of dry weight
3E	N 2 hrs.	.1478	.0389	26.4	.010	6.7
C105	N 2 days	.1329	.0395	29.7	—	—
26	N 5 days	.3725	.0960	26.5	.019	5.1
124	N 7 days	.1811	.0564	31.0	.017	9.1
39	D 1 day	.2678	.1042	39.3	.010	3.7
20b	D 4 days	.3692	.1385	37.4	.041	11.0
13	D 7 days	.1474	.0502	35.0	.010	6.8
49	D 7 days	.3015	.1098	36.5	.031	10.2
35b	D 12 days	.2552	.0528	20.7	.013	5.2
31	D 17 days	.3417	.1034	30.2	.021	6.2
15	D 18 days	.3406	.1213	32.5	—	—

Destalked animals, on the contrary, did increase in carapace width and exhibited this increase soon after ecdysis. Table VIII contains values for carapace widths of destalked animals before and after ecdysis, and the percentage increase over the width before ecdysis. For purposes of comparison similar data are presented for the six normal animals. It will be seen, therefore, that at least for a postmolt period of seven days, destalked animals present a striking contrast to normal animals.

4. Hardening

Table IX contains data for the inorganic composition of a group of postmolt animals, including both normal and destalked. One of the most notable differences between the two is the relatively high ash content of destalked forms. It now becomes of interest to examine the composition of the cast exuviae of destalked and

TABLE X

Relationship in destalked animals of the ash and calcium contents of the exuviae to the interval between destalking and ecdysis. Exuviae of two normal animals are included for comparison

Exuvia no.	Days after destalking that animal underwent ecdysis	Dry weight (gms.)	Ash		Calcium	
			(gms.)	% of dry weight	(gms.)	% of dry weight
3E	2 hours	.2430	.1233	50.0	.052	21.5
A18	5 days	.2228	.1409	63.5	—	—
44	6 days	.1919	.0919	48.0	—	—
9	6 days	.2056	.1084	53.0	.058	28.2
A19	7 days	.1570	.0704	44.0	—	—
13	8 days	.1704	.0830	49.0	.047	27.8
30	10 days	.2698	.1296	48.0	.076	28.6
A26	10 days	.2530	—	—	.073	29.0
6	27 days	.3279	.1571	47.0	—	—
41	27 days	.3939	.1919	48.0	—	—
55	Normal animals	.4396	.2584	59.0	.122	27.7
5F		.2324	.1444	64.0	—	—

normal animals. This information is given in Table X. Indicated are the dry weights, ash weights, calcium, and the ash and calcium as percentages of the dry weights. It will be seen that animals which undergo ecdysis within five or six days after destalking shed an exuvia which contains about 53% ash; if ecdysis occurs later than six days after destalking, the ash content is about 47%. This value of 47% is found for exuviae of destalked animals which are cast between six and 27 days after destalking. In contrast, normal animals shed an exuvia which contains about 61% ash. The percentage of calcium, however, is about 27% for exuviae from both normal and destalked animals. That the calcium content of exuviae from destalked animals is about 27% was reported by Kleinholz and Bourquin (1941b). It will be seen by an inspection of Tables IX and X that the extent of the reduction in ash content in exuviae of destalked animals, in comparison with normal, corresponds closely with the proportionally higher ash content which is observed in destalked postmolt animals, a value of approximately 10% having been obtained in both cases.

DISCUSSION

One of the effects of bilateral eyestalk removal in *Uca pugilator* has been shown through these investigations to be a reduction of the non-calcium inorganic content of the exoskeleton, and the apparent transfer of this material into the soft tissues of the animal (*cf.*, Tables IX and X). Since the calcium content of the exoskeleton is not appreciably altered by eyestalk ablation, it would appear that the sinus gland-X organ complex exerts a specific influence not on calcium metabolism *per se*, but rather on processes which control anionic metabolism. Such a mode of action would affect the calcium level in an indirect manner. In all probability a similar situation occurred in Koller's (1930) investigations of *Crago*; he reported that the acid-solu-

TABLE XI

Calculations in terms of various forms of calcium for normal and destalked U. pugilator

Animal no.	Number of days after ecdysis	Condition	Calcium		A as % of ash	B as % of ash
			A calculated as 100% calcium oxide	B calculated as 100% calcium phosphate		
39	1	Destalked	.014	.020	13.4	19.2
20b	4	Destalked	.057	.084	41.0	60.8
13	7	Destalked	.014	.020	28.0	40.0
49	7	Destalked	.043	.063	39.4	57.6
35b	12	Destalked	.018	.026	34.1	49.2
31	17	Destalked	.029	.043	28.0	41.6
3E	0	Normal	.014	.020	36.0	51.2
26	5	Normal	.026	.039	27.0	40.7
124	7	Normal	.024	.035	42.5	62.0
			(values obtained)*		(ash calculated)**	
C112	Not known	Normal	.031	.017	50.0	27.5
C111	Not known	Normal	.018	.008	40.5	17.0
D29	Not known	Normal	.096	.039	53.3	23.0
D31	Not known	Normal	.078	—	42.6	—

* From Table IV.

** Since actual ash weights could not be determined (a limitation of the procedure), they have been calculated by the formula:

$$\frac{\text{ash weight}}{\text{dry weight}} = 0.32$$

ble mineral content of cast exuviae from destalked animals was less than that found in cast exuviae of normal animals. Furthermore, the results of Kleinholz and Bourquin (1941b), indicating no difference between exuviae of normal and destalked *Palaemonetes* with respect to the calcium contents, may be attributable to a similar mode of action.

A consideration of the anionic composition of animals is necessarily limited; quantitative determinations for phosphate and carbonate were made only on normal animals. Nevertheless, it is possible to suggest certain limitations which are imposed by assuming that calcium is bound primarily only to one or the other, or both, of these anions under all conditions. In Table XI calculations are presented for

the phosphate and carbonate contents of a series of normal and destalked animals; these calculations are based on the ionic calcium values which were actually obtained (Tables IV and IX), expressed as oxides of calcium carbonate and calcium phosphate. In each case it was assumed that the calcium is present entirely in the form of these two compounds. In addition, the calculated oxides are expressed as percentages of the ash.

An examination of Table XI indicates that approximately 70% of the ash can be attributed to the oxides of these compounds; about 46% is from carbonate and 23% comes from phosphate. The remaining 30% of the ash probably consists of oxides of silicon, magnesium, aluminum, iron and sulfur since these elements normally occur in crustacean cuticles (Clark and Wheeler, 1922). If the calcium content of *Uca pugilator*, seven days after ecdysis, is assumed to be entirely of calcium carbonate, the oxides which are obtained by incineration will account for 43% of the ash; if the calcium content is assumed to consist completely of calcium phosphate, the resulting oxides would account for 62% of the ash. In contrast to this situation, destalked animals which were analyzed 17 days after ecdysis showed a maximum of 28% ash from carbonate and a maximum of 41% ash from phosphate. From these facts, it would appear quite clear that some other elements were responsible for the remaining ash. If the calcium compound were considered as calcium sulfate, it would account for approximately 70% of the ash. It is known that the sulfur content of lobsters is considerably higher in older animals than in younger animals (Richards, 1951). The possibility exists that the unknown ash component of destalked, postmolt animals contains a high percentage of sulfur; further investigations on the nature of the ash are needed and may be of considerable significance in attempting to explain the role of the sinus gland in mineral metabolism.

That there is an alteration of the calcium:phosphorus ratio in freshly molted animals as compared to premolt animals has been shown by Robertson (1937); his results with *Carcinus maenas* show the integument of normal, freshly molted animals to be considerably higher in phosphorus than in premolt animals. Expressing his values as the ratio of the percentage of calcium to the percentage of phosphorus, it is seen that premolt animals have a ratio of about 9.5 as compared with values of 0.9 shortly after ecdysis, and 1.7 within a week after ecdysis. On the basis of calculations in Table XI, it is not unlikely that a similar situation may occur in *U. pugilator*.

The results of Edwards (1950) with *U. pugnax* are interpreted by that investigator as positive evidence for an increase in weight subsequent to bilateral eyestalk removal. Of a total of ten animals used for one experiment, six showed a weight increase within two days after the operation. However, if the percentage changes in weights of these six animals are expressed as the averages for two, three and seven days after destalking, increases of 3.0%, 3.0% and 5.5%, respectively, are obtained. In contrast, and not discussed by Edwards, similar calculations for animals which showed a decrease in weight after destalking indicate average decreases of 0.5%, 14.4%, 6.3% and 9.3% at the end of one, two, three and eight days, respectively. Yet, on the basis of the data, Edwards concludes that eyestalk removal leads to an increase in weight.

An inspection of Table V might lead one to believe that destalked animals increase in weight. At the end of six, seven, nine and ten days, the percentage increases for five animals are, respectively, 0.7%, 1.6%, 0.3% and 1.9%. However, it should be remembered that the daily fluctuations of wet weights are sufficient to

account for at least a 2% variation which may be considered either as an increase or decrease of the wet weight. While the range of fluctuations in weight of a destalked animal is less than that of a normal animal, it is possible to demonstrate similar variations. Until additional studies conducted over a greater period of time are made, it seems necessary to conclude that eyestalk removal has had little effect on the wet weight of *U. pugilator*, at least during a ten-day period following the removal.

That water metabolism is in some manner influenced by a hormone source in the eyestalk has some experimental verification. Data are inadequate for a thorough consideration of this aspect; nevertheless, they suggest a possible role of the sinus gland controlling the rhythmical fluctuation in weight. Normal postmolt animals appear to exhibit a diurnal fluctuation in wet weight (Figs. 7a and 7b). Normal premolt animals also show diurnal fluctuations, but as ecdysis is approached, the fluctuations appear to become arrhythmic. Although destalked premolt animals show minor fluctuations, they were not of a rhythmical character. It will be recalled that these rhythmical fluctuations in wet weight were observed only in animals which were kept in running sea water at the Marine Biological Laboratory. Such a rhythm was not observed in animals which were kept in finger bowls with either sea water or distilled water; under these conditions the sea water was subject to evaporation, while neither medium was aerated to the extent of normally running sea water. These differences may well account for the observed absence of a rhythm under these conditions.

An hypothesis of a rhythm in water-uptake, controlled by the sinus gland-X organ complex and with the phase of wet-weight change as described in this report, would suggest a daily fluctuation in blood calcium concentration of normal animals. If such were the case, it would support an assumption made by Edwards (1950), although the mechanism of control would not be that suggested by Edwards. Finally, it would explain why a more pronounced rhythm is seen in normal postmolt animals than in animals immediately prior to ecdysis, since it is generally thought that the titer of sinus gland hormone is lower just before ecdysis than after ecdysis.

It was observed that normal animals showed no increase in carapace width during a ten-day period following ecdysis, in contrast to the situation in destalked forms (Table VIII). The apparent failure of normal animals to assume greater dimensions during the immediate postmolt period has a number of possible explanations:

1. The environmental factors which prevail in the normal molting habitat may be significantly different from those existing in the laboratory, and may be favorable for a growth response. Normally, animals in the field presumably have an adequate food supply at their disposal; such may not have been the case in the laboratory, since the only food available to the animals was that which was brought in by the running sea water. Changes in the chemical composition of sea water in the salt marshes, effected by drainage, may well exist and be responsible for differences from the conditions in the sea water brought into the laboratory. Following summer rains, a hypotonic condition might have an effect on the amount of water which is absorbed by an animal at ecdysis. The sea water which is pumped into the laboratories at the Marine Biological Laboratory comes from an area which is not subject to any appreciable variation.

2. Since the yearly cycle for *U. pugilator* has not been observed, a situation may exist which is similar to that of certain crayfish. Scudamore (1947) found that

Cambarus underwent two yearly molts, growth occurring to a much greater extent in the late summer molt than in the spring molt. Inasmuch as the frequency of molting in normal animals was extremely low during the July-August period, there may well be normally an early summer molt at which time animals exhibit substantial increases in carapace width.

3. Another possibility, and one which is realized in some crustaceans, is that a dimensional increase following ecdysis may be considerably reduced or even lacking under certain conditions. Lloyd and Yonge (1947) found that the growth rate of *Crangon vulgaris* declines as the animals approach maturity and that females which carried eggs showed no increase in length after ecdysis. Hoglund (1943) reported that molt without growth occurs in *Leander squilla* during the winter. Inasmuch as the normal animals on which measurements were taken during the present investigations probably represent adult individuals, it is possible that they had already attained their maximum dimensions. If such an hypothesis is correct, then any additional molts which might occur after such maximal dimensions have been established may serve only to facilitate the regeneration of lost appendages or the repair of damaged portions of the cuticle.

SUMMARY

1. A transitional series of color changes which occurs during the premolt period of normal animals is described. These changes have been arbitrarily assigned to five stages and the duration of each stage measured. The last four of these are associated with hypodermal activity anticipating ecdysis.

2. The inorganic constituents of normal and destalked animals are compared for the premolt and postmolt condition.

3. Certain physical aspects of ecdysis are treated in a quantitative manner. These include the absorption of water and dimensional changes.

4. The results suggest that the sinus gland-X organ complex plays a role in the regulation of water metabolism and in the metabolism of inorganic constituents.

5. Evidence is presented for a diurnal rhythm of water-uptake under the control of the sinus gland-X organ complex.

6. The possibility that molt and dimensional increase are separate factors under certain conditions is discussed.

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